

REMARKS

Claim Amendments

Claim 1 has been amended to specify the pH of the media in steps (c)-(g). Support for this language can be found at page 11, lines 4-5 of the specification. Claim 1 has also been amended to specify that the suspension culture is performed for about 10 to about 14 days. Support for this language can be found at page 9, lines 9-12 of the specification. Claim 1 has further been amended to specify the concentrations of asparagine and glutamine in the medium of step (g). Support for this language can be found at page 10, lines 14-18. Finally, claim 1 has been amended to specify that the asparagine and glutamine replaces ammonium nitrogen in the medium of step (g). Support for this language can be found at page 11, lines 8-10 of the specification.

Claim 8 has been amended to depend from claim 9 and to make it clear that the source of nitrogen relates to the medium of step (g). Support for this language can be found in claims 7-9 as originally filed.

Claim 9 has been amended to make it clear that the source of nitrogen relates to the medium of step (g). Support for this language can be found in claims 7-9 as originally filed.

Claim 10 has been amended to make it clear that the source of nitrogen relates to the medium of step (g). Support for this language can be found in claims 7-10 as originally filed.

In view of the amendment to claim 1, claim 13 has been canceled, and claim 14 has been amended accordingly.

Claim 19 has been amended to specify the pH of the media in steps (c)-(g). Support for this language can be found at page 11, lines 4-5 of the specification. Claim 19 has also been amended to specify that the suspension culture is performed for about 10 to about 14 days. Support for this language can be found at page 9, lines 9-12 of the specification. Claim 19 has further been amended to specify the concentrations of asparagine and glutamine in the medium of step (g). Support for this language can be found at page 10, lines 14-18. Finally, claim 19 has been amended to specify that the asparagine and glutamine replaces ammonium nitrogen in the medium of step (g). Support for this language can be found at page 11, lines 8-10 of the specification.

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Amendment dated 21 January 2009
Reply to Office Action dated 19 September 2008

Claim 22 has been amended to make it clear that the medium refers to the medium of step (h). Support for this language can be found at page 9, lines 24-27.

Claim 25 has been amended to make it clear that the medium refers to the medium of step (h). Support for this language can be found at page 9, lines 24-27.

Claim 27 has been amended to make it clear that the source of nitrogen relates to the medium of step (g). Support for this language can be found in claims 7-9 as originally filed.

In view of the amendment to claim 19, claim 29 has been canceled.

In view of the amendments to claims 1 and 19, claims 31-36 have been canceled.

It is submitted that these amendments do not constitute new matter, and their entry is requested.

The Invention

The present invention is directed to a method of producing transgenic cotton plants which has been developed for the generation of transgenic cotton plants of many different cotton varieties and to increase the transformation efficiency and survival rate. As disclosed in the present application, the steps and the media have been developed and selected to maximize the efficiency of regeneration of *Agrobacterium* transformed cotton cells. *See*, pages 9-11 of the instant application. The method involves the use of specified plant hormones only in the callus induction medium with no plant hormones being used in any of the other media. The method also involves the use glucose as the sole carbon source in all of the media except the plant growth medium which utilizes both glucose and sucrose. The method further involves the use of a short, i.e., about 10-14 days, suspension culture step to induce formation of embryogenic calli. No embryoids are produced in this short time. Finally, the method involves the use of asparagine and/or glutamine as a nitrogen source in place of ammonium nitrogen in the embryoid germination medium. The method of the present invention has been developed to look after the requirements for embryogenesis of transformed tissue at the various stages of the process.

The various elements set forth in the claims have been found to provide a cotton transformation system that has a higher transformation efficiency and higher rate of survival to produce a higher frequency of transformed plants than what was obtained with prior art methods. For example,

It was found that shortened period of suspension culture treatment [about 10 to about 14 days] is important for high frequency induction of embryogenesis. It is also important for reducing production of abnormal embryos, since a high percentage of vitreous embryos that are poor in regeneration are produced when cotton calli are maintained in suspension culture for too long (Chen et al., unpublished observation).

See, page 9, lines 12-19 of the specification. Similarly,

... it has been found that asparagine and glutamine were better nitrogen source than inorganic ammonia nitrogen for supporting embryoid development and root development. In the preferred MMS3 medium (described below), which contains asparagine and glutamine as the nitrogen source, the growth of non-embryogenic calli was inhibited while the germination, growth and root development of embryoids were preferentially promoted. Because of healthy root development, the survival rate of potted transgenic cotton plants obtained by the methods of the present invention is almost 100%.

See, page 11, lines 15-26 of the specification. It is the combination of all of the elements of the claimed subject matter that enables a higher transformation frequency and higher rate of survival with the consequent higher frequency of transformed plants. The use of all of the claimed elements is not suggested by the prior art.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1-2, 4-5, 8-11, 13-14, 18-20, 22-23, 25, 27 and 30-36 under 35 U.S.C. § 103(a) as being obvious over Rangan (5,244,802) in view of Gawel et al. (*Plant Cell Tissue Organ Develop* **23**:201-204, 1990), further in view of Price et al. (*Plant* **145**:305-307, 1979) and further in view of Tuli et al. (US 6,242,257). Applicants traverse this rejection.

The Examiner cites Rangan for disclosing a process in which (I) callus is induced from cotyledon or hypocotyl explants on a medium containing glucose, kinetin and NAA, (ii)

embryogenic callus is formed by growing the callus on medium containing a cytokinin and NAA, (iii) embryogenic callus is developed in suspension culture using a medium containing sucrose and NAA over 5 to 36 days, (iv) embryoids are formed on a solid medium and (v) the embryoids are germinated on a medium containing 500 mg/l casein hydrolysate (which is a source of both asparagine and glutamine) and 1.2 g/l KNO_3 . The Examiner also cites Rangan for disclosing transformation of the explants by exposing the explants to *Agrobacterium* in a medium containing NAA. The Examiner notes that Rangan does not teach the use of petioles as the explant, the use of 2,4-D for callus initiation, the use of suspension culture in embryogenic callus formation, the lack of hormones in the exposing, selection, embryogenic callus formation and embryoid formation media and the use of 3.8 KNO_3 and/or 500 mg/l asparagine and/or 1 g/l glutamine in the germinating medium.

The Examiner cites Price et al. for disclosing the culturing of callus in a hormone free suspension culture to induce formation of embryogenic callus and embryoids. The Examiner cites Gawel et al. for disclosing the use of cotton petiole as the explant, the use of 2,4-D and kinetin for callus initiation and the culturing of callus in suspension culture to induce formation of embryogenic callus and embryoids in which suspension culture was preferable to a solid medium. The Examiner cites Tuli et al. for the use of glucose as the sole carbon source in all media and the use of both glucose and sucrose as the carbon source in the “regenerating media.” The Examiner contends that it would have been obvious to modify the modified Rangan process (as discussed above) to use glucose as the sole carbon source in all media or to use both glucose and sucrose in the “regenerating media.”

In view of these references, the Examiner contends that it would have been obvious to modify the transformation method of Rangan to (I) use petioles as the explant, (ii) use a suspension culture in the embryogenic callus formation step, (iii) use media lacking hormones in the exposing, selection, embryogenic callus formation and embryoid formation steps, (iv) use 3.8 g/l KNO_3 and/or 500 mg/l asparagine and/or 1 g/l glutamine in the germinating medium and (v) use glucose as the sole carbon source in all media or use both glucose and sucrose as the carbon source in the

regenerating medium. The Examiner also contends that it would have been obvious to try a pH of 6.2 to 7.0, because they are close to cellular pHs.

Applicants initially submit that the Examiner's characterization and interpretation of Rangan is not entirely correct. For example, the Examiner asserts that Rangan utilizes 1.2 g/l KNO_3 in the germinating medium. However, Rangan does not utilize KNO_3 but instead utilizes 1.2 g/l NH_4NO_3 . *See*, the Beasley and Ting medium composition in the table at the bottom of column 6 - top of column 7. This medium also contains 1.01 g/l KNO_3 based on the directions for preparing the stock solution and the amount of stock solution to use. This amount is significantly less than that used (3.8 g) in the present invention. Thus, Applicants submit that the Examiner's characterization of the germinating medium is not correct.

In addition, the Examiner asserts that embryogenic calli can be developed in suspension culture over about 5 to 36 days. Despite providing specific reference to the teachings of Rangan with respect to other elements of Rangan's disclosed method, the Examiner does not provide any specific reference to a disclosure in Rangan that refers to 5 to 36 days for the development of embryogenic calli. Instead, the growth is for about 3 to 4 weeks. After this time, the suspension culture is filtered to remove large cell clumps and then further cultured for 3 to 4 weeks. The large cell clumps were cultured on callus growth medium to produce somatic embryos. *See*, Example 6. The steps of filtering and further culturing can be repeated. Furthermore, Applicants have performed a word search of Rangan and have not been able to locate any such specific teaching. If the Examiner is of the opinion that Rangan contains such a disclosure, Applicants request that the Examiner provide a specific citation to such teaching in Rangan. In the absence of any such specific citation, Applicants submit that the Examiner's characterization of the suspension culture is not correct.

Furthermore, Applicants submit that the suspension culture of Rangan is for proliferating the embryogenic callus and not for inducing formation of embryogenic callus as the Examiner has also asserted. Specifically, the Examiner also contends that it is the nonembryogenic callus that is used as the starting material by Rangan for initiating suspension cultures, because Rangan "consistently

refers to nonembryogenic callus as ‘callus’ and embryogenic callus as ‘embryogenic callus.’” Although Rangan et al. does appear to use the terms fairly consistently as noted by the Examiner, Applicants submit that a proper reading of the disclosure of Rangan as a whole clearly teaches that the starting material used by Rangan for initiating the suspension cultures is embryogenic callus. For example, column 3, lines 48-52 disclose that it is the embryogenic callus that is cultured in suspension. The heading at column 9, lines 46-47 discloses that embryogenic cells are proliferated in suspension, which can only mean that embryogenic cells are initiated into suspension culture. The description at column 9, lines 48-51 concerning further developing embryogenic callus, which is expressed as an “alternative,” can only mean that the embryogenic callus is grown in suspension culture as an alternative to growing the embryogenic callus on solid medium as described in the specification prior to this description. This description must further be interpreted to have this meaning in view of the heading title immediately preceding this description. Column 10, lines 14-17 also discloses that the embryogenic callus is used to initiate suspension cultures. Examples 6 and 7, which are examples describing the suspension cultures of (a) column 9, line 47 - column 10, line 13 and (b) column 10, lines 13-31, respectively, disclose the use of embryogenic callus as the starting material for the suspension cultures. Finally, claims 16 and 17 directed to methods using a suspension culturing step utilize the embryogenic callus as the source of callus for initiating the suspension cultures. In addition, Rangan et al. clearly discloses that the growth of embryogenic callus in suspension requires NAA. Thus, Applicants submit that a correct interpretation of the teachings of Rangan is that embryogenic callus is added to the suspension culture and proliferated in suspension culture. Since Rangan teaches the use of embryogenic callus for initiating suspension cultures, there is no disclosure in Rangan of culturing callus tissue in suspension culture to induce embryogenic callus formation. There is also no disclosure in Rangan of using a culture time of about 10 to about 14 days for the inducement of embryogenic callus formation in suspension culture. Thus, Rangan lacks this element of the claimed subject matter.

The present specification clearly discloses the advantages of using a short suspension culture for inducing embryogenic callus. See, for example, page 9, lines 12-19. This advantage is not

taught or suggested in Rangan. Thus, Applicants submit that there is no teaching or suggestion in Rangan to use a short suspension culture step, i.e., about 10 to about 14 days, to induce the formation of embryogenic calli. In fact, Applicants submit that Rangan teaches away from such a step because it specifically teaches using embryogenic callus for the initiation of suspension culture and culturing the embryogenic callus for 3-4 week periods.

The Examiner notes that Rangan teaches the use of casein hydrolysate, which is a source of asparagine and glutamine, in the germination medium. Applicants note that the medium used by Rangan contains 1 g/l KNO_3 , 1.2 g/l NH_4NO_3 and is preferably supplemented with 500 mg/l casein hydrolysate. Although the germination medium of Rangan may be supplemented with casein hydrolysate, there is no teaching or suggestion in Rangan to use asparagine and/or glutamine in place of inorganic ammonium nitrogen, e.g., in place of NH_4NO_3 , as claimed. There also is no teaching or suggestion in Rangan to use the amounts of asparagine and/or glutamine set forth in the claims, which are not present in such amounts in the specified amount of casein hydrolysate used by Rangan. The present specification clearly discloses the advantages of using asparagine and/or glutamine in place of inorganic ammonium nitrogen. See, for example, page 10, lines 11-21. Furthermore, Applicants submit that there are numerous examples in the literature that a specific amino acid or a combination of a few amino acids may differ from casein hydrolysate in modulation of physiological processes. For example, one of the present Applicants has published a paper reporting the different effects of casein hydrolysate and amino acids on antibiotics production (Zhang et al., *J Applied Microbiology* **85**, 1023-1028, 1998). The difference could be due to the quantity of active amino acids or due to inhibitory substance in casein hydrolysate. Thus, Applicants submit that there is no teaching or suggestion in Rangan to use asparagine and/or glutamine in place of inorganic ammonium nitrogen in the claimed amounts. In fact, Applicants submit that Rangan teaches away from such a replacement because Rangan utilizes casein hydrolysate in addition to inorganic ammonium nitrogen.

In addition, according to Rangan, callus is induced from hypocotyls and/or cotyledons on a first callus medium that contains NAA and kinetin as plant hormones and glucose as a carbon

source. The callus is then subcultured on the first callus growth medium. Subsequently, the callus is cultured on a second callus growth medium. The second callus growth medium contains NAA and optionally cytokinin as plant hormones. Rangan specifically teaches that glucose is used as the carbon source until the secretion of phenolics has ceased after which sucrose is used as the carbon source in this second callus growth medium. The callus is subcultured on the second callus growth medium to produce embryogenic callus and somatic embryos. *See*, column 8, line 20 - column 9, line 13. Interestingly, Rangan specifically teaches that

[T]he essential difference between the first and second callus growth medium is the carbon source. Glucose is used during period of high phenolic secretions. Sucrose is used when secretion have stopped.

See, column 8, lines 63-66. Similarly, the suspension culture which is based on the second callus growth medium contains sucrose. *See*, column 9, lines 54-55 and column 13, lines 7-12. Applicants submit that Rangan teaches using sucrose as the carbon source for the initiation of embryogenic callus as well as in all media other than the first callus growth medium. Thus, Applicants submit that Rangan specifically teaches away from using glucose as the sole carbon source in all of the media as set forth in claims 1 and 19.

Rangan teaches that hormones are used in the first callus growth medium, the second callus growth medium and the suspension culture medium. Specifically about 2 mg/l NAA and 1 mg/l kinetin are used in the first callus growth medium (column 8, lines 20-29), 1-10 mg/l NAA and 0-1 g/l cytokinin (column 8, lines 55-60) are used in the second callus growth medium and about 2 mg/l NAA (column 10, lines 14-17) is used in the suspension culture medium. In addition, Rangan teaches that hormones are also used in the same media for plant transformation. *See*, column 10, line 36 to column 11, line 2. Thus, Applicants submit that Rangan teaches away from not using hormones in all media subsequent to callus initiation.

Gawel et al. only relates to somatic embryogenesis and does not describe a method that includes transformation. Gawel et al. discloses inducing callus tissue on a medium that contains either (1) NAA and kinetin or (2) 2,4-D and kinetin as the plant hormones. Callus produced on

either of these media were then cultured either on a semi-solid medium or in suspension culture using an “embryo proliferation medium” which is hormone-free. The culturing was for 8 weeks. Gawel et al. discloses that the suspension culture was preferable for the production of somatic embryos. According to Gawel et al., the initiation medium (i.e., callus induction medium) supported embryogenesis, i.e., callus and embryogenic callus is induced on the same medium, and thus plant hormones were used for both callus induction and embryogenic callus induction. *See*, page 202, left column top. Consequently, the callus cultured in suspension culture was embryogenic callus and the suspension culture was not used to induce the formation of embryogenic callus. Furthermore, since Gawel et al. uses 8 weeks for culturing, Applicants submit that there is no teaching or suggestion to use a short suspension culture step, i.e., about 10 to about 14 days, to induce the formation of embryogenic calli. Thus, Applicants submit that Gawel et al. teaches away from such a step because it specifically teaches using embryogenic callus for the initiation of suspension culture and culturing the embryogenic callus for 8 weeks.

In addition, Applicants submit that there is no motivation to combine Gawel et al. with Rangan because the two references are culturing different materials in suspension, i.e., callus for Gawel et al. and embryogenic callus for Rangan, which also explains the different times for the suspension culturing. That is, Rangan et al. use 3-4 weeks before removing cell clumps, whereas Gawel et al. uses 8 weeks before removing embryos. The present invention only uses a short suspension culturing step to induce embryogenic callus formation and not to induce embryo formation which is not obvious from the combined teachings of Rangan and Gawel et al.

Price et al. only relates to somatic embryogenesis and does not describe a method that includes transformation. Price et al. teaches using IAA and kinetin as plant hormones for the induction of callus on a medium. The callus tissue was then subcultured on a medium containing 2iP and NAA as plant hormones to produce very friable, vigorously growing callus cultures. These callus cultures were used to initiate suspension cultures. Price et al. specifically teaches that 2iP and NAA are required in the subculturing step in order to obtain somatic embryogenesis in suspension culture. *See*, page 306, left column. The suspension cultures either contained 2,4-D as a plant

hormone or lacked the presence of 2,4-D. According to the abstract this culturing is for 3-4 weeks, although the methods indicate culturing for 6-14 days and then filtering through a cheese cloth. In addition, Price et al. teaches that suspension cultures initiated without 2,4-D were not vigorous and formed only two embryoids. Thus, Price et al. specifically teaches that 2,4-D is required for increased vigor of the suspension culture and high frequency formation of embryoids. *See*, page 306, right column. Certainly if a high frequency of embryoids is the goal, a skilled artisan would utilize 2,4-D necessary for initiating the suspension culture in view of the teachings of Price et al. Thus, Applicants submit that Price et al. teaches away from the use of no hormones in the suspension culture step because very few embryoids are obtained.

Price et al. also teaches the use of asparagine or more particularly glutamine in the suspension culture and is essential in inducing somatic embryogenesis. Price et al. supplements the B5 medium with asparagine or glutamine. The B5 medium still contains the inorganic ammonium nitrogen. The asparagine or glutamine does not replace this inorganic ammonium nitrogen. Thus, Applicants submit that Price et al. teaches away from replacing inorganic ammonium nitrogen with asparagine or glutamine. Furthermore, Price et al. use asparagine or glutamine in the suspension culture step. There is no teaching in Price et al. to use them in the germination medium.

In addition, Applicants note that Price et al. was published eight years prior to the filing date of Rangan et al. Applicants submit that the fact that Rangan et al. did not use the techniques of Price et al. in their invention is very telling that a skilled artisan would not have been motivated to use the teachings of Price et al. with Rangan. Furthermore, Applicants submit that even if Price et al. could be combined with Rangan with respect to modifying the germination medium to contain asparagine or glutamine, the combination would still require the presence of inorganic ammonium nitrogen that both Rangan and Price et al. utilized.

Finally, Applicants submit that the Examiner's characterization of one of the teachings of Price et al. is not correct. On page 8 of the Office Action, the Examiner contends that Price et al. does not show that 2iP and NAA are required for somatic embryogenesis. The Examiner contends that Price et al. did not teach whether 2iP or other cytokinins were required but suggested testing

if that was the case. On the contrary, Price specifically teaches that 2iP and NAA were required in the subculturing step, the step between callus initiation and suspension culture initiation, to obtain somatic embryogenesis. *See*, page 306, left column. It was only the concentration of 2iP that Price et al. suggested testing. *See*, page 306, right column. Thus, Applicants submit that the Examiner's characterization of the lack of requirement of 2iP for somatic embryogenesis in Price et al. is not correct.

Tuli et al. relates to a process for the organogenesis of cotton. Applicants submit that it is well known in the art that organogenesis and embryogenesis are two entirely different techniques for generating plants in tissue culture, as confirmed by Tuli et al. which states,

Organogenesis leads to organ formation i.e., shoot (or root), which can be isolated to induce development of roots (or shoots) to produce full plant while somatic embryogenesis leads to the development of somatic embryos (embryos developed without fertilization) which have both shoot and root initials and are capable of developing into whole plant.

See, column 1, lines 60-66. The skilled artisan knows that techniques that are useful for one technique are not necessarily useful for the other technique. In fact, Tuli et al. states,

Although the ability of individual parts of plants and cells to regenerate into complete plants (called totipotency) is a well-known phenomenon, each plant or plant part requires specialized studies to invent the conditions that allow such regeneration. Some of the broadly applicable factors controlling growth and differentiation of such cultures have been determined. The establishment of interactions among different groups of phytohormones and growth regulators alone or in combinations are responsible for certain interrelations existing among cells, tissues and organs. There seems to be consensus that the success in inducing differentiation depends upon the type of explant, physiological condition of the explant and physical and chemical milieu of the explant during culture. Due to this, the science of tissue culture has been directed to optimize the physiological conditions of source plant, the type of explant, the culture conditions and the phytohormones used to initiate tissue culture. **This substantiates the fact that development of a new process for proliferation of plants by tissue culture is not obvious.**

See, column 1, line 66 - column 2, line 18 (emphasis added).

In view of the well-known differences between organogenesis and embryogenesis, Applicants submit that a skilled artisan would not combine Tuli et al. with the other cited references as the Examiner has done, especially in view of the explicit disclosure in Tuli et al. for the desire to develop an efficient process for the generation of a large number of shoots from a new tissue explant.

In addition, Rangan et al., which is somatic embryogenesis, specifically teaches that glucose is used in the culture medium during the period of phenolic secretions and that sucrose is used once phenolic secretions have ended. However, Tuli et al. teaches that there is a higher amount of phenolics leaching out on a medium containing sucrose or a combination of sucrose and glucose in organogenesis cultures. Thus, the use of glucose in the medium was preferred by Tuli et al. for culturing the explant to produce multiple shoots. These teachings are in direct contrast to each other. Interestingly, the methods of Rangan et al. are included in Tuli et al. in Table 1 which shows the state of the art of cotton tissue culture. This art was distinguished by Tuli et al. in the description of their invention. Since Rangan et al. was known to Tuli et al. and a different technique entirely was developed to produce multiple shoots by organogenesis, Applicants submit that this fact is evidence that a skilled artisan would not have used methods of organogenesis for producing transgenic plants by somatic embryogenesis.

The Examiner also contends at page 5 of the Office Action that it would have been obvious to try a higher pH than those used in the prior art because it would be closer to cellular pH. Applicants submit that this contention is not proper, particularly in view of the prior art which specifically teaches otherwise. Specifically, Applicants note that Rangan uses a pH of 5.8-6.0, Gawel et al. uses a pH of 5.5 and Tuli et al. uses a pH of 5.4-6.2. Price et al. is silent as to the pH of its media, however, typically the pH of MS medium is 5.8 and the pH of B5 medium is 5.5. This cited art specifically teaches using pH below 6.2 and thus Applicants submit teaches away from the use of the pH specified in the present claims.

To establish a *prima facie* case of obviousness, the Patent Office must establish that the prior art included each element claimed (M.P.E.P. 2143). In addition, “[a] patent composed of several

elements is not proved obvious merely by demonstrating that each element was, independently, known in the prior art." *KSR International Co. v. Teleflex Inc.*, 167 L. Ed. 2d 705, 712, 82 USPQ2d 1385, 1389 (2007). The Supreme Court in *KSR* reaffirmed the familiar framework for determining obviousness as set forth in *Graham v. John Deere Co.* (383 U.S. 1, 148 USPQ 459 (1966)), but stated that the Federal Circuit had erred by applying the teaching-suggestion-motivation (TSM) test in an overly rigid and formalistic way. However, although a rejection need not be based on a teaching or suggestion to combine, a preferred search will be directed to finding references that provide such a teaching or suggestion if they exist, especially where it is clear that the claimed invention is not a simple substitution, predictable extension or anticipated result of the prior art at the time of filing. M.P.E.P. 2141. Under section 103, "[b]oth the suggestion and the expectation of success must be founded in the prior art, not in applicant's disclosure" (*Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 1207, 18 USPQ2d 1016 (Fed. Cir. 1991), quoting *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed Cir. 1988)). Moreover, when a combination of references are used to establish a *prima facie* case of obviousness, the Patent Office must present evidence that one having ordinary skill in the art would have been motivated to combine the teachings in the applied references in the proposed manner to arrive at the claimed invention. *See, e.g., Carella v. Starlight Archery*, 804 F.2d 135, 231 USPQ 644 (Fed. Cir. 1986); and *Ashland Oil, Inc. v. Delta Resins and Refractories, Inc.*, 776 F.2d 281, 227 USPQ 657 (Fed. Cir. 1985).

Because of the well-known issue of plant cell viability following *Agrobacterium* infection, Applicants do not believe that a skilled artisan would reasonably expect that the simple application of somatic embryogenesis techniques could be readily combined with transformation protocols to derive a transformation such as the Examiner has done in the formulation of the present rejection. The very art cited by the Examiner shows the unpredictability in the art. For example, Price et al. teaches that subculturing on a medium containing 2iP and NAA is absolutely required in order to obtain embryoids in the subsequent suspension culture. Price et al. also shows that glutamine is required in the medium used to produce somatic embryoids from the suspension cultured material.

Thus, the combination of Price et al. with Rangan would require the use of 2iP and NAA in a method which includes suspension culture in order to achieve embryoid formation. The presently claimed subject matter does not use such hormones as taught by Price et al. Thus, Applicants submit that the amended claims are not obvious from the prior art cited by the Examiner.

Furthermore, the very art cited by the Examiner teaches away from the present invention and from making the combination of art as made by the Examiner in the present rejection. Specifically, Applicants submit that Rangan teaches away from a short suspension culturing step to induce the formation of embryogenic calli, because Rangan specifically teaches using embryogenic callus for the initiation of suspension culture and specifically teaches culturing the embryogenic callus for 3-4 week periods. Similarly, Applicants submit that Gawel et al. teaches away from short suspension culturing step to induce the formation of embryogenic calli, because Gawel et al. specifically teaches using embryogenic callus for the initiation of suspension culture and specifically teaches culturing the embryogenic callus for 8 weeks.

Also, Applicants submit that Rangan teaches away from replacing the inorganic ammonium nitrogen with asparagine and/or glutamine in the germination medium, because Rangan specifically teaches using casein hydrolysate in addition to inorganic ammonium nitrogen. Similarly, Price et al. teaches away from replacing the inorganic ammonium nitrogen with asparagine or glutamine in the suspension culture, because Price et al. specifically teaches adding asparagine or glutamine to the medium which includes inorganic ammonium nitrogen. In addition, Price et al. only teaches using asparagine or glutamine in the suspension culture and does not teach about using it in the germination medium.

In addition, Applicants submit that Rangan specifically teaches away from using glucose as the sole carbon source in all of the media as set forth in the claimed invention, because Rangan specifically teaches using glucose only until the secretion of phenolics has subsided and then using sucrose. Rangan et al. specifically teaches using sucrose as the sole carbon source in the suspension culture.

Furthermore, Applicants submit that Rangan teaches away from not using hormones in all media subsequent to callus initiation, because Rangan specifically teaches using hormones in the callus growth media and in the suspension culture. Similarly, Price et al. teaches away from not using hormones in all media subsequent to callus initiation, because Price et al. specifically teaches using hormones in the callus initiation medium, the callus subculturing medium, and the suspension culture, the latter of which was required for higher frequency of embryoid formation, i.e., higher than two.

Thus, it is clear that the combination of the prior art does not teach all of the elements of the presently claimed invention. It is also clear that the prior art specifically teaches away from the claimed subject matter. In view of the teaching away by the cited prior art, there is no motivation to make any of the changes to the process of Rangan as made by the Examiner to the present rejection. Thus, Applicants submit that the present invention is not obvious from the teachings of Rangan, Gawel et al., Price et al. and Tuli et al.

In addition, Applicants submit that the combination of the references proposed by the Examiner would not lead to the present invention even if there was some motivation to make the combination. For example, the combination of the teachings of Rangan, Gawel et al. and Price et al. does not lead to step (e) of claims 1 and 19 in which a suspension culture step is performed for only about 10 to about 14 days to induce the formation of embryogenic calli. The cited references all use a suspension culture step that is longer than this specified time. In addition, the combination of the teachings of Rangan, Gawel et al. and Price et al. does not lead to the use of hormones in only the callus initiation step, because they use hormones in callus initiation and in suspension culture, especially for high frequency embryoid formation in the case of Price et al. Thus, Applicants submit that the present invention is not obvious from the teachings of Rangan, Gawel et al., Price et al. and Tuli et al.

In view of the above amendments and remarks, Applicants submit that Rangan, Gawel et al., Price et al. and Tuli et al. does not render obvious the claimed subject matter. Withdrawal of this rejection is requested.

Application Serial No. 10/009,590
Amendment dated 21 January 2009
Reply to Office Action dated 19 September 2008

Conclusion

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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